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Shih-Pin Huang
Institute of Marine Biology, National Taiwan Ocean University, Keelung, Taiwan, R.O.C.

I-Shiung Chen
Institute of Marine Biology, National Taiwan Ocean University, Keelung, Taiwan, R.O.C.

Kwang-Tsao Shao
Biodiversity Research Center, Academia Sinica, Taipei, Taiwan, R.O.C, zoskt@gate.sinica.edu.tw

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THE MOLECULAR PHYLOGENY OF GENUS *Hemigobius* (TELEOSTEI: GOBIIDAE), WITH THE CONFIRMATION OF VALIDITY OF *Hemigobius crassa* (HERRE, 1945)

Shih-Pin Huang[1], I-Shiung Chen[1], and Kwang-Tsao Shao[2]

Key words: *Hemigobius*, mangrove, gobies, Taiwan.

ABSTRACT

The genus *Hemigobius* Bleeker, 1874 is has been regarded as only two valid species in previous study including *H. hoevenii* (Bleeker, 1851) and *H. mingi* (Herre, 1936). Traditionally, the both *Vaimosa crassa* Herre, 1945 and *Mugilogobius obliquifasciatus* Wu and Ni, 1985 found from Chinese and Taiwanese waters had ever been regarded as the junior synonyms of *H. hoevenii*. However, our present study have been confirm that *H. crassa* collected from Hong Kong and Taiwan is a valid species. The *H. crassa* can be well distinguished from *H. hoevenii* based on several morphological features. The molecular phylogenetic analysis firstly based on the mitochondrial partial sequences of ND5, complete Cyt-b genes and D-loop region. The distinct mitogenetic differentiation has been observed between both *H. hoevenii* and *H. crassa*. The phylogenetic trees also reveal that *H. crassa* and *H. hoevenii* are sister group, and *H. mingi* belong to another distant clade.

I. INTRODUCTION

The genus *Hemigobius* is established by Bleeker in 1874 [3], which is based on type species as *H. hoevenii* (Bleeker, 1851) [2] collected from Borneo. The genus *Hemigobius* species widely distributed in Indo-west Pacific region, which is mostly in brackish water habitat [4, 21, 34]. *H. hoevenii* is widely distributed over in Thailand, Singapore, Indonesia, Philippines, Papua New Guinea and Australia [20, 21], and this species also has been reported in Taiwan and China [4, 34]. *H. mingi* (Herre, 1936) [15] is distributed around tropical areas in Thailand, Singapore and Indonesia [20, 21].

There are two possible *Hemigobius* species have been described in southern China. The *Vaimosa crassa* Herre, 1945 collected from Hong Kong, and *Mugilogobius obliquifasciatus* Wu and Ni, 1985 collected from Hainan Island, Goungdong and Fujian Province, southern China. Previous studies consider they should be considered that two species are the junior synonyms of *Hemigobius hoevenii* [20, 21]. On the other hand, the *Hemigobius bleekeri* Koumans, 1953 [19] collected from Java and *Sphenentogobius vanderbilti* Fowler, 1940 [12] collected from Sumatra also be regarded as synonym of *H. mingi* [20, 21]. So far, merely two species including *H. hoevenii* and *H. mingi* would be regarded as valid member of genus *Hemigobius* in previous studies [20, 21].

Our survey for gobioid fish fauna from more recently collections including the *H. hoevenii* and *H. mingi* collected from Malay Peninsula, and also captured the so-called *H. hoevenii* from mangroves of Hong Kong and western Taiwan, and we consider the *H. hoevenii* and the so-called *H. hoevenii* from Hong Kong and Taiwan possess minor, but conspicuous morphological differentiations, thus, our study focused on those specimens collected from Hong Kong and Taiwan employing both morphological and molecular approaches.

The aims of theses papers are not only documenting the real species diversity of the genus, but also providing the comments on the molecular phylogenetic relationship reconstructed by molecular sequence data.

II. MATERIALS AND METHODS

1. Sample Collections

All the examined *Hemigobius* specimens collected from Malay Peninsula, Hong Kong and Taiwan were collected by hand-net. The specimens used for molecular analysis were preserved in 95% ethanol; other remaining specimens used for morphological studies were fixed in 10% formalin directly before being transferred into 70% ethanol for long-term preservation.

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[1] Institute of Marine Biology, National Taiwan Ocean University, Keelung, Taiwan, R.O.C.
[2] Biodiversity Research Center, Academia Sinica, Taipei, Taiwan, R.O.C.

2. Morphological Studies

Morphometric methods follow Miller (1988) [24] and meristic methods follow Akihito *et al*. (1984), Chen and Shao (1996), Chen *et al*. (1999), Chen and Kottelat (2003), Chen and Miller (2008) and Huang and Chen (2007) [1, 5-8, 17]. Terminology of cephalic sensory canals and free neuromast organs (sensory papillae) is from Wongrat and Miller (1991) [32], based on Sanzo (1911) [29]. All examined materials are deposited at the Institute of Marine Biology, National Taiwan Ocean University, Keelung, Taiwan (NTOU).

Meristic abbreviations are as follows: A, anal fin; C, caudal fin; D1 and D2, first and second dorsal fins, respectively; LR, longitudinal scale series; P, pectoral fin; PreD, predorsal scales; SDP, scale series from origin of first dorsal fin to upper pectoral origin; TR, transverse scale series from second dorsal to anal fin; VC, vertebral count. All fish lengths are standard length (SL).

3. Molecular Phylogenetic Analysis

The phylogenetic relationships are employed the mtDNA sequence of full length of Cytochrom b (Cyt-b), D-loop and partial mitochondrial NADH dehydrogenase subunit 5 (ND5) in this study. All DNA extractions of the samples were using a kit (Roche, High Pure Product Preparation kit). Cyt b region were amplified by polymerase chain reaction (PCR) using following two primers: (GGluF: 5'-TAACCAGGACTARTG RCTTG-3'; GProR: 5'-GTTARAATCTCYYTTCTTTGA-3'); D-loop region were amplified by polymerase chain reaction (PCR) using following two primers: (GTHR: 5'-TCAGCGCC AGAGCGCCGKTCTTGTAA-3'; PGL5: 5'-CTAGGGYCTA TCCTAACATCTTCA-3'); ND5 region were amplified by PCR using following two primers: (PGleuD1: 5'-AAAGGAT AACAGCTCATCCGTTGGTCT-3'; ND5MR: 5'-CCTATTT TKCGGATGTCYTG-3').

PCR was done in a MODEL 2700 or 9700 thermal cycler (Perkin-Elmer) and 30-40 cycles were carried out. The 50 μL reaction volume contained 33.5 μL of sterile distilled water, 5 μL of 10X PCR buffer (Takara), 4 μL of dNTP (2.5 mM each), 3 μL of Mgcl2 (2.5 mM each), 1 μL of each primer, 0.5 μL of 0.5 unit Ex *Taq* (Takara) and 2 μL of template. The thermal cycler profile was as follows: denaturation at 94°C for 60 seconds, annealing at 52-58°C for 60 seconds and extension at 72°C for 120 seconds. A negative control without template was carried out for each run of PCR. The PCR products were run on a 1.0% L 03 agarose gel (Takara) and stained with ethidium bromide for band characterization under ultraviolet trans-illumination.

Double-stranded PCR products were purified using a kit (Roche, High Pure Product Purification kit), before undergoing direct cycle sequencing with dye-labeled terminators (ABI Big-Dye kit). The sequencing primers used were same as PCR using primers. All sequencing reactions were performed according to the manufacturer's instructions. Labeled fragments were analyzed using as ABI PRISM Model 377-64 DNA Automated sequencer (ABI).

Nucleotide sequence alignment was verified manually after running through BIOEDIT version 5.9 [14]. The analysis of aligned mutation sites were conducted using Molecular Evolutionary Genetics Analysis (MEGA) version 5.05 [31] for aligned mutation sites analysis.

The parsimony (MP) analysis was carried out using PAUP* version 4.0B10 [30] using heuristic search. Branch support was established via bootstrap analysis (2000 replications). For the Bayesian (BI) analysis, the best-fitting model for sequence evolution was determined for mtDNA D-loop and ND5 sequences using MrMODELTEST version 2.2 [26]. The BI analyses were performed using MrBayes 3.0 [28]. The posterior probabilities of each node were computed from remaining 75% of all sampled trees.

III. RESULTS

1. Molecular Phylogenetic Analysis

The aligned Cyt-b, D-loop and ND5 sequence consists of 6 different haplotypes and from all 3 *Hemigobius* species with 8 individuals, the outgroup was assigned as *Pseudogobius javanicus* (Bleeker, 1856), which is one of related genera of *Hemigobius*. The length of combined sequence of Cy-b, ND5 and D-loop sequence is 3011-3012 bp in total (1141 bp in Cyt-b, 835-836 bp in D-loop, and 1035 bp in ND5). This alignment contain 647 total number of mutations, and 586 number of polymorphic (segregating) sites. The phylogenetic analysis using neighbour-joining (NJ), parsimony (MP) anlysis and Bayesian inference (BI) method provided. The phylogenetic tree was reconstructed by NJ analysis based on Kimura 2-parameter model. The phylogenetic tree reconstructed by BI analysis based on HKY 85+G model.

The result of MP analysis by heuristic search only one tree, and tree length 1082; the Consistency index (CI) being 0.9325, Retention index (RI) being 0.7944 and Homoplasy index (HI) being 0.0675.

The phylogenetic trees reconstructed by NJ, MP and BI methods shows that same grouping result. The phylogenetic trees congruently reveal that *H. crassa* and *H. hoevenii* are sister group, and *H. mingi* belong to another distant clade, All specific level of nodes with high bootstrap value reach to 100 in NJ tree, 92-100 in MP tree and posterior probabilities as high as 100 in BI tree, the specific level of nodes are strongly supported.

In comparison with mitogenetic diversity of interspecific relationship among all *Hemigobius* species, the range of mitogenetic divergence is from 8.5-19.1% based on combined Cyt-b, ND5 and D-loop sequences; the intra-specific genetic divergence are 0.7-0.9% between the *H. carssa* collected from Taiwan and Hong Kong based on combined Cyt-b, ND5 and D-loop sequences. The range of interspecific mitogenetic divergence of all *Hemigobius* species are from 10.2-21.5%, 10.8-25.4% and 3.4-9.4% for Cyt-b, ND5 and D-loop sequences respectively based on K2P model.

Table 1. Sampling localities, OTU codes and accession number of molecular sequence analysis of 3 *Hemigobius* species and outgroup from Taiwan, Hong Kong and Malay Peninsula.

Code	Species	Locality	Accession number		
			Cytb	ND5	D-loop
HCRPZ1	*Hemigobius crassa*	Estuary of Putzu River, Dongshi Township, Chiayi County, Taiwan	KF851359	KF851363	KF779935
HCRPZ2	*Hemigobius crassa*	Estuary of Putzu River, Dongshi Township, Chiayi County, Taiwan	KF851360	KF851363	KF779936
HCRHK1	*Hemigobius crassa*	Mangrove of Hong Kong, China	KF851361	KF851364	KF779937
HCRHK2	*Hemigobius crassa*	Mangrove of Hong Kong, China	KF851361	KF851365	KF779938
HHOML1	*Hemigobius hoevenii*	Matang mangrove, Malaysia	KF851357	KC995183	KC995177
HMIML1	*Hemigobius mingi*	Matang mangrove, Malaysia	KF851358	KF851362	KF779934
PJAKM1	*Pseudogobius javanicus*	Mangrove of Liehyu Island, Kinmen County, Taiwan	KF193873	KF193873	KF193873

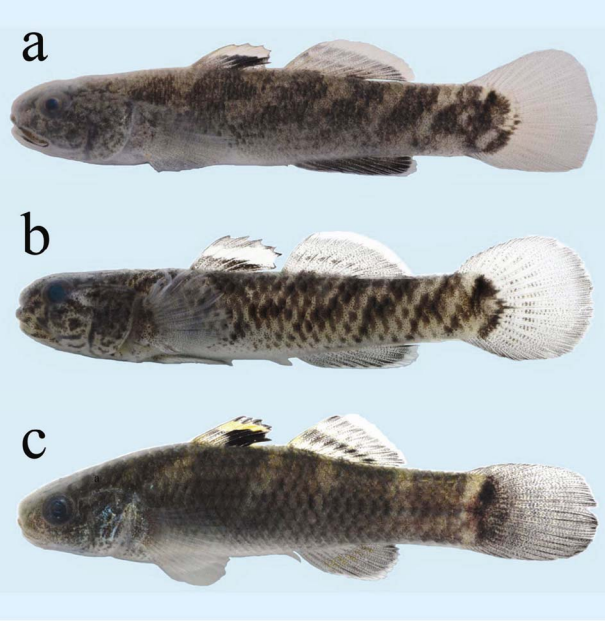

Fig. 1. The specimen photographs of 3 *Hemigobius* species from Taiwan and Malay Peninsula. a, *Hemigobius crassa*, NTOUP 2012-09-156, male, 30.1 mm SL. b, *Hemigobius hoevenii*, NTOUP 2011-05-003, male, 24.5 mm SL. c, *Hemigobius mingi*, NTOUP 2011-05-008, male, 29.0 mm SL.

IV. SYSTEMATICS

***Hemigobius crassa* (Herre, 1945)**

(Table 2; Fig. 1a, 3a, 4)

Vaimosa crassa Herre, 1945: 403 (brook near Un Long, Hong Kong) [16].

Mugilogobius obliquifasciatus Wu and Ni, 1985: 93 (Haikou, Hainan Island, China). 1986: 272-273 [33].

Material examined:

NTOUP 2012-09-153, 2 specimens, 16.2-16.5 mm SL, mangrove of Hong Kong, China, coll. I-S. Chen, 18 July, 2012.

NTOUP 2012-09-154, 4 specimens, 21.4-23.9 mm SL, estuary of Puzi River, Dongshi Township, Chiayi County, Taiwan, coll. I-S. Chen, November, 1995. NTOUP 201209-155, 1 specimen, 24.6 mm SL, estuary of Putzi River, Dongshi Township, Chiayi County, Taiwan, coll. S. P. Huang, 12 August, 2012. NTOUP 2012-09-156, 4 specimens, 28.1-30.1 mm SL, estuary of Putzi River, Dongshi Township, Chiayi County, Taiwan, coll. S. P. Huang, 20 August, 2012. NTOUP 2012-10-157, 4 specimens, 20.6-29.6 mm SL, mangrove of Kinmen Island, Taiwan, coll. I-S. Chen, 6 October, 2012.

Diagnosis

Hemigobius crassa can be well distinguished from other congeners by the unique combination of following features: (1) fin rays: D2 I/7-8 (modally 8), A I/8, P 16-18 (modally 17). (2) squamation: longitudinal scale rows 33-35 (modally 34), transverse scale series 11-12 (modally 11), predorsal scales 12-15 (modally 13), and cheek naked. (3) specific coloration: Body with 6 lateral, conspicuous and oblique blackish brown blotches, the marks about 1.5-2 times of width of their interspaces. Chin in lacking any bar or spot. First dorsal fin with a broad horizontal yellow margin in adult male, and white in female. Upper caudal fin base with a small pale yellow spot. Caudal fin membrane has no any line or spot.

Redescription

Body elongate, subcylindrical anteriorly and compressed posteriorly. Head large and anterior region compressed. Snout slightly prominent than the lower lip. Eye rather large. Mouth medium sized, male slightly bigger than female, maxillary extending to the vertical of anterior margin of pupil in male, but only maxillary extending to the vertical of midline of anterior margin of orbit and anterior margin of pupil in female. Anterior nasal as short tube, posterior nasal as round hole. Gill-opening extending ventrally forward the middle vertical line of operculum. VC 10 + 16 = 26 (in 4).

Fins.-D1 VI; D2 I/7-8 (modally 8); A I/8; P 16-18 (modally 17). First dorsal fin low and rounded, spines never filamenttous; third to fourth spines always longest, the spine is slightly longer in male than female, and they can not extending to anterior edge of second dorsal when pressed in both sexes.

Table 2. Morphometric measurements of 3 *Hemigobius* species from Taiwan, Hong Kong and Malay Peninsula.

	H. crassa		*H. hoevenii*		*H. mingi*	
	Male 5	Female 2	Male 6	Female 6	Male 4	Female 2
Percent standard length (%)						
Head length	26.7 – 28.5 (27.7)	26.1 – 26.4 (26.3)	26.0 – 28.1 (27.2)	24.9 – 26.7 (25.8)	25.6 – 27.1 (26.4)	25.3 – 25.6 (25.5)
Predorsal length	37.8 – 39.2 (38.3)	37.9 – 39.0 (38.5)	38.1 – 40.2 (39.6)	38.0 – 39.9 (38.8)	37.3 – 39.7 (38.7)	39.2 – 40.2 (39.7)
Snout to 2nd dorsal origin	54.9 – 56.1 (55.4)	55.4 – 56.4 (55.9)	55.2 – 59.5 (57.2)	55.8 – 58.1 (57.5)	54.6 – 58.0 (56.4)	56.0 – 57.1 (56.5)
Snout to anus	51.7 – 53.2 (52.3)	52.1 – 53.6 (52.9)	53.3 – 56.2 (55.3)	54.4 – 57.2 (55.1)	55.7 – 55.9 (55.2)	57.7 – 58.2 (58.0)
Snout to anal fin origin	56.9 – 58.6 (57.9)	58.9 – 59.7 (59.3)	57.7 – 61.4 (60.1)	59.7 – 61.7 (60.5)	56.6 – 61.2 (59.1)	62.1 – 62.5 (62.3)
Prepelvic length	28.0 – 29.0 (28.4)	27.9 – 29.0 (28.5)	28.9 – 30.7 (30.3)	27.6 – 29.0 (28.3)	28.4 – 29.5 (29.0)	29.2 – 31.0 (30.1)
Caudal peduncle length	30.0 – 32.2 (30.9)	30.5 – 30.7 (30.6)	25.5 – 29.9 (27.5)	25.3 – 29.8 (27.1)	28.8 – 31.2 (30.4)	29.2 – 31.5 (30.4)
Caudal peduncle depth	14.0 – 14.4 (14.2)	13.2 – 14.2 (13.7)	14.9 – 15.7 (15.2)	14.2 – 16.7 (15.1)	13.8 – 15.5 (14.8)	14.2 – 14.9 (14.5)
1st dorsal fin base	10.1 – 10.4 (10.3)	9.3 – 9.6 (9.5)	9.9 – 10.4 (10.2)	9.6 – 10.7 (10.0)	10.8 – 12.9 (11.9)	11.9 – 12.5 (12.2)
2nd dorsal fin base	15.8 – 16.4 (16.1)	13.4 – 13.9 (13.6)	16.3 – 17.2 (16.9)	15.3 – 16.2 (15.7)	13.0 – 15.6 (14.4)	14.5 – 15.7 (15.1)
Anal fin base	14.5 – 16.9 (15.5)	14.4 – 15.4 (14.9)	14.6 – 15.5 (15.1)	14.5 – 15.1 (14.7)	10.7 – 12.3 (11.7)	12.0 – 12.6 (12.3)
Caudal fin length	20.3 – 21.2 (20.8)	18.8 – 19.6 (19.2)	20.8 – 24.0 (22.2)	20.1 – 22.0 (21.3)	25.5 – 26.9 (26.1)	25.9 – 26.7 (26.3)
Pectoral fin length	19.8 – 20.5 (20.1)	19.5 – 20.5 (20.0)	22.3 – 24.1 (23.2)	22.1 – 24.0 (23.4)	19.1 – 23.0 (21.2)	19.6 – 20.8 (20.2)
Pelvic fin length	13.4 – 14.9 (14.2)	14.1 – 15.7 (14.9)	14.3 – 16.6 (15.1)	14.2 – 17.0 (16.6)	17.0 – 17.6 (17.3)	15.8 – 17.5 (16.7)
Body depth at pelvic fin origin	17.0 – 19.1 (18.0)	18.0 – 18.5 (18.3)	16.0 – 18.5 (16.8)	16.2 – 17.3 (16.9)	19.2 – 20.6 (19.8)	19.4 – 20.9 (20.2)
Body depth at anal fin origin	17.2 – 18.7 (18.0)	17.8 – 18.0 (17.9)	16.0 – 17.5 (16.5)	16.3 – 17.6 (16.8)	18.7 – 21.2 (19.8)	20.0 – 20.3 (20.1)
Body width at anal fin origin	14.2 – 15.1 (14.7)	15.8 – 16.1 (15.9)	12.9 – 14.7 (13.8)	12.5 – 13.7 (13.2)	9.9 – 11.7 (10.7)	10.2 – 11.2 (10.7)
Pelvic fin origin to anus	23.3 – 24.0 (23.6)	26.1 – 26.4 (26.2)	23.8 – 25.5 (24.3)	26.1 – 28.3 (27.2)	28.1 – 28.6 (28.3)	29.2 – 30.2 (29.7)
Percent head length (%)						
Snout length	33.4 – 36.6 (34.7)	31.0 – 31.9 (31.4)	35.7 – 39.1 (37.5)	32.3 – 35.3 (34.5)	31.0 – 32.5 (31.6)	30.0 – 30.2 (30.1)
Eye diameter	24.2 – 26.8 (25.3)	28.1 – 29.9 (29.0)	26.7 – 28.5 (27.6)	28.2 – 30.5 (29.4)	27.0 – 30.2 (28.9)	31.9 – 32.7 (32.3)
Cheek depth	29.0 – 31.3 (30.4)	26.0 – 26.2 (26.1)	30.3 – 32.6 (31.5)	30.1 – 32.3 (31.8)	23.2 – 26.0 (24.9)	24.1 – 25.3 (24.7)
Postorbital length	46.7 – 49.5 (48.0)	49.5 – 49.9 (49.7)	43.2 – 45.2 (44.2)	43.4 – 46.9 (45.3)	42.3 – 45.0 (43.1)	43.2 – 44.0 (43.6)
Head width in maximum	73.6 – 77.8 (75.7)	76.5 – 77.7 (77.1)	81.3 – 85.3 (83.6)	79.8 – 82.1 (80.6)	68.9 – 73.2 (72.3)	69.8 – 71.2 (70.5)
Head width in upper gill	54.9 – 56.5 (55.6)	55.2 – 54.3 (54.7)	56.1 – 58.5 (56.9)	55.2 – 57.9 (56.8)	50.6 – 55.2 (52.3)	53.7 – 55.1 (54.4)
Bony interorbital width	27.7 – 28.4 (28.1)	28.3 – 29.8 (29.1)	28.9 – 32.4 (30.2)	29.1 – 31.5 (30.7)	30.0 – 32.3 (31.4)	29.3 – 31.6 (30.5)
Fleshy interorbital width	39.1 – 41.5 (40.3)	42.2 – 42.7 (42.6)	42.7 – 43.9 (43.1)	40.9 – 43.0 (42.0)	44.5 – 46.0 (45.5)	46.1 – 47.3 (46.7)
Lower jaw length	43.1 – 47.3 (45.7)	39.0 – 40.2 (39.6)	45.6 – 48.0 (46.9)	40.4 – 43.7 (41.6)	36.1 – 39.7 (37.8)	33.8 – 35.3 (34.5)

Anal fin inserted below first branched rays of second dorsal fin. Pelvic fin medium sized and rounded. Caudal fin rounded.

Scales.-LR 33-35 (modally 34); TR 11-12 (modally 11); PreD 12-15 (modally 13); SDP 10-11 (modally 10). Body cover with ctenoid scales. Predorsal region covered with cycloid scales. Belly covered with smaller cycloid scales. Cheek naked. Operculum covered with many small cycloid scales.

Head lateral-line system

Head canals- Head pores present. There is a pair of pore α in upper region of rear edge of orbit, and a pair of λ, κ and ω distribute over on between two eye region.

Sensory papillae- Cheek with typical longitudinal papilla pattern. Row *a* hort, about two-thirds of orbit diameter. Row *b* with densely-set papillae, and about two-thirds of orbit diameter. Row *c* merely with single papilla. Row *cp* short, about half of orbit diameter. and Row *d* short, about one-thirds of orbit diameter. Row *s* with two row papillae. Row *p* incomplete. Opercular rows with rows *os*, *oi* and *ot*. Rows *oi* and *ot* slightly separated. Rows *f* with pair papillae.

Coloration in life

Head and trunk with pale yellowish brown or pale grayish brown background. Cheek with a broad grayish black oblique stripe starting from rear edge of orbit extending to preoperculum. A blackish brown stripe starting from neck and across orbit extending to snout. Lower operculum with a vertical grayish black stripe and extending to ventral operculum. Chin in lacking no any bar or spot. Lateral body with 6 conspicuous and oblique blackish brown blotches, the marks about 1.5-2 times width of their interspaces. Upper pectoral fin region with an oblique brownish black band extending to predorsal region, lower caudal fin base with an oblique blackish brown band extending to central caudal fin base. Upper caudal fin base with a small pale yellow spot in adult both sexes. Pectoral fin membrane pale grayish white, base a horizontal grayish black bar. First dorsal fin with a broad horizontal yellow margin in adult male, and white in female, and a broad horizontal grayish black blotch under the margin. Second dorsal fin with a broad horizontal gray blotch, and with broad pale yellow margin in both sexes. Ventral fin grayish black in male, and pale grayish white in female. Anal fin grayish

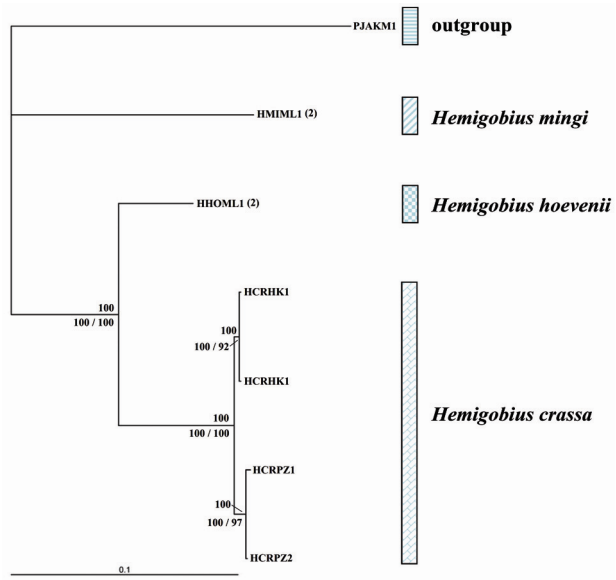

Fig. 2. Molecular phylogenetic tree of *Hemigobius* species from Taiwan, Hong Kong and Malay Peninsula based on combined Cyt-b, D-loop and partial ND5 sequence reconstructed by Bayesian analysis method based on the Kimura HKY 85+G model (values above the branch: posterior probability). The similar topology for bootstrap consensus tree by neighbour-joining method (anterior value) and maximum parsimony method (posterior value) list only the bootstrap (value below the branch: bootstrap number, 2000 replications).

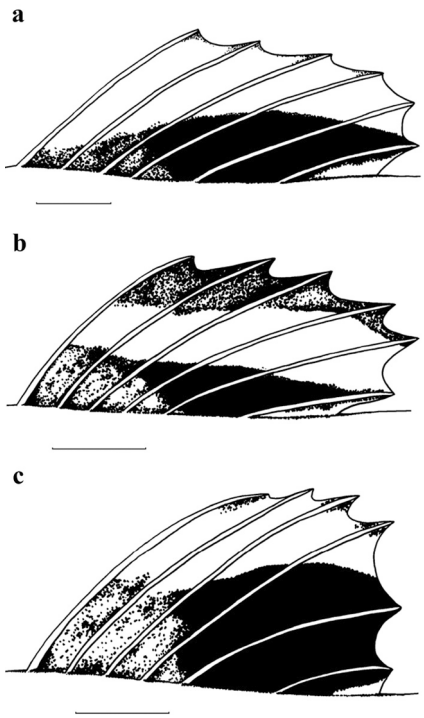

Fig. 3. The color pattern of first dorsal fin of 3 *Hemigobius* species from Taiwan and Malay Peninsula. a, *Hemigobius crassa*, male, 30.1 mm SL. b, *Hemigobius hoevenii*, male, 24.5 mm SL. c, *Hemigobius mingi*, male, 29.0 mm SL. Bar = 1 mm. Drawing by Shih-Pin Huang.

black in adult male, and pale grayish in female, and with white margin in both sexes. Caudal fin membrane pale grayish white, and has no any spot or line in both sexes.

The morphological comparison of *H. crassa* and *H. hoevenii*

Although both *H. crassa* and *H. hoevenii* share high similarity for overall lateral pigmentation pattern, our data are strongly supporting that *H. crassa* is discrete species, we can well distinguish both *H. crassa* and *H. hoevenii* based on following morphological features: (1) H. crassa possess more fin rays: D2 I/7-8 (modally 8), A I/8, P 16-18 (modally 17) *vs.* D2 I/7, A I/7, P 15-17 (modally 16); (2) H. crassa possess more scale series: LR 33-35 (modally 34), TR 11-12 (modally 11), PreD 12-15 (modally 13-14) *vs.* LR 28-30 (modally 29), TR 9-10 (modally 9), PreD 10-12 (modally 11); (3) different color patterns: *H. crassa* body blotches about 1.5-2 times of their interspaces; chin without any bars; caudal fin membrane without any line or spot, and first dorsal fin with a broad horizontal yellow margin in adult male, and white in female *vs.* *H. hoevenii* with lateral blotches as wide as their interspaces; chin with many vermiform bars; caudal fin membrane with 5-9 vertical black lines, and first dorsal fin with a broad horizontal grayish black margin.

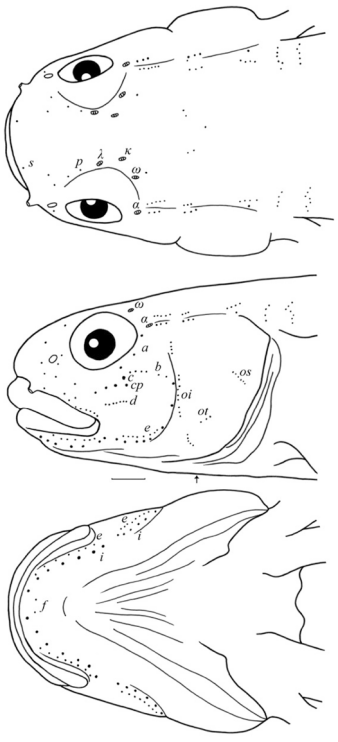

Fig. 4. Head lateral-line system of *Hemigobius crassa* from Taiwan. NTOUP 2012-09-156, male, 30.1 mm SL. Bar = 1 mm. Drawing by Shih-Pin Huang.

***Hemigobius hoevenii* (Bleeker, 1851)**

(Table 2; Fig. 1b, 2b, 3b, 5)

Table 3. Frequency distribution of meristic features of of 3 *Hemigobius* species from Taiwan, Hong Kong and Malay Peninsula.

	D1		D2			A			P						
	VI	x	I/7	I/8	x	I/7	I/8	x	13	14	15	16	17	18	x
H. crassa	14	6.0	1	13	7.9	–	14	8.0	–	–	–	5	15	2	16.9
H. hoevenii	25	6.0	25	–	7.0	25	–	7.0	–	–	13	32	5	–	15.8
H. mingi	6	6.0	6	–	7.0	6	–	7.0	2	9	–	–	–	–	13.8

	LR									TR				
	28	29	30	31	32	33	34	35	x	9	10	11	12	x
H. crassa	–	–	–	–	–	9	16	3	33.8	–	–	10	4	11.3
H. hoevenii	1	29	20	–	–	–	–	–	29.4	14	11	–	–	9.4
H. mingi	–	1	8	3	–	–	–	–	30.2	–	6	–	–	10.0

	PreD									SDP							VC	
	8	9	10	11	12	13	14	15	x	6	7	8	9	10	11	x	26	x
H. crassa	–	–	–	–	3	5	4	2	13.4	–	–	–	–	11	3	10.2	4	26.0
H. hoevenii	–	–	8	13	4	–	–	–	10.8	–	–	14	10	1	–	8.5	12	26.0
H. mingi	2	4	–	–	–	–	–	–	8.7	1	5	–	–	–	–	6.8	4	26.0

Gobius hoevenii Bleeker, 1851: 426 (Sambas, in river, Borneo) [2].

Stigmatogobius hoevenii: Koumans, 1953: 125 [19].

Hemigobius hoevenii: Kottelat *et al*., 1993: 146; Lim and Larson, 1994: 259; Larson, 2001: 74 (in part); Larson and Lim, 2005: 109 [18, 21, 22, 23].

Material examined:

NTOUP 2011-05-003, 23 specimens, 19.3-26.2 mm SL, Matang mangrove, Malaysia, coll. I-S. Chen and S. P. Huang, 20 April, 2011. NTOUP 2011-05-016, 7 specimens, 14.4-22.9 mm SL, Matang mangrove, Malaysia, coll. I-S. Chen and S. P. Huang, 21 April, 2011.

Diagnosis

Hemigobius hoevenii can be well distinguished from other congeners by the unique combination of following features: (1) fin rays: D2 I/7, A I/7, P 15-17 (modally 16); (2) squamation: longitudinal scale rows 28-30 (modally 29), transverse scale series 9-10 (modally 9), predorsal scales 10-12 (modally 11), and cheek naked; (3) specific coloration: body side with 6 conspicuous and oblique blackish brown blotches, the marks as wide as their interspaces. Chin with many vermiform bars. First dorsal fin with a broad horizontal grayish black margin. Upper caudal fin base with a small pale yellow spot. Caudal fin membrane with 5-9 vertical black lines in both sexes.

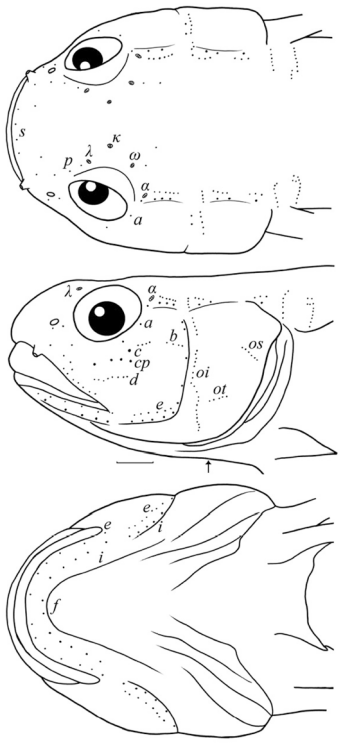

Fig. 5. Head lateral-line system of *Hemigobius hoevenii* from Malay Peninsula. NTOUP 2011-05-003, male, 24.5 mm SL. Bar = 1 mm. Drawing by Shih-Pin Huang.

***Hemigobius mingi* (Herre, 1936)**

(Table 2; Fig. 1c, 2c, 3c, 6)

Gnatholepis mingi Herre, 1936: 8 (Pulau Ubin, Singapore) [15].

Sphenentogobius vanderbilti Fowler, 1940: 396 (Medan, Sumatra) [12].

Stigmatogobius mingi: Koumans, 1953: 118 [19].

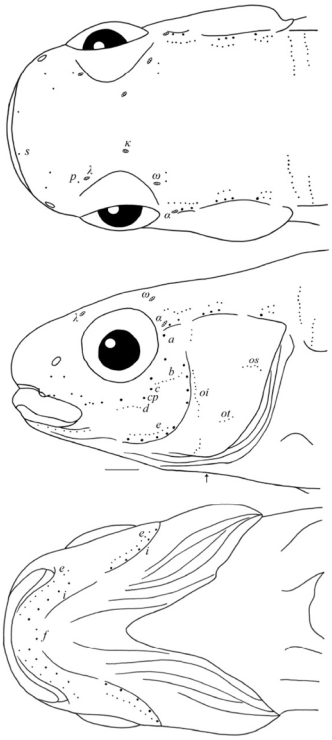

Fig. 6. Head lateral-line system of *Hemigobius mingi* from Malay Peninsula. NTOUP 2011-05-008, male, 29.0 mm SL. Bar = 1 mm. Drawing by Shih-Pin Huang.

Hemigobius bleekeri Koumans, 1953: 191 (replacement name for *Gobius melanurus* Bleeker, 1849) [19].

Hemigobius mingi: Lim and Larson, 1994: 259; Larson, 2001: 74; Larson and Lim, 2005: 110 [21, 22, 23].

Material examined:

NTOUP 2011-05-008, 5 specimens, 25.9-28.9 mm SL, Matang mangrove, Malaysia, coll. I-S. Chen and S. P. Huang, 20 April, 2011.

Diagnosis

Hemigobius mingi can be well distinguished from other congeners by the unique combination of following features: (1) fin rays: D2 I/7; A I/7; P 13-14 (modally 14); (2) squamation: longitudinal scale rows 29-31 (modally 30), transverse scale series 10, predorsal scales 8-9 (modally 9), and cheek covered with scale; (3) specific coloration: body light yellow with 4 conspicuous and very broad brownish black bands; transverse blackish brown marks about 2-3 times of width of their interspaces; first dorsal fin with a large rounded black blotch; caudal fin base with a vertical broad blackish brown band; caudal fin membrane grayish black, and with 2-3 irregular vertical black lines in both sexes.

V. DISCUSSION

The *M. obliquifasciatus* Wu and Ni, 1985 should be regarded as junior synonym of *H. crassa* based on the survey from China and Taiwan and it is highly possible that *H. crassa* may only distribute in subtropical West Pacific of Eastern Asia including southern China, Taiwan and even northern Vietnam.

The genetic divergence of *H. crassa* and other *Hemigobius* species are from 3.4-9.4% for D-loop sequences based on the K2P model**,** it is higher than the divergence 2.6-3.8% between Taiwanese freshwater cyprinid, *Opsariichthys pachycephalus* (Günther, 1868) [13] and *Opsariichthys kaopingensis* Chen and Wu, 2009 [10], and also higher than the 3.5-3.6% between *Candidia barbatus* (Regan, 1908) [27] and *Candidia pingtungensis* Chen, Wu and Hsu, 2008 [9] based on mitochondrial DNA D-loop sequence [9, 10]. In comparison with that of marine fish, the genetic divergence of *H. crassa* and other *Hemigobius* species (3.4-9.4%) is even higher than Atlantic coral-reef wrasse, *Thalassoma ascensionis* and *T. sanctaehelenae* (3.2%) for D-loop sequences based on K2P model [11].

The genetic divergence of *H. crassa* and other *Hemigobius* species are from 10.8-25.4% for ND5 sequences based on K2P model**,** it is also higher than those among Japanese Yoshinobori (*Rhinogobius* spp.) from 4.0 % to 4.8 % based on K2P model [25].

The mitogenetic divergence have firstly addressed and discussed for genus *Hemigobius* or related genus in previous studies, thus this study will contribute to define the further understanding of the molecular phylogeny, taxonomic status within the higher level of related taxonomic ranks.

Diagnostic key to nominal species of *Hemigobius* species of the Indo-West Pacific:

1. Pectoral fin rays 13-14; predorsal scales 8-9; lateral body with 4 very broad bands; caudal fin base with a vertical broad blackish brown band ····· *H. mingi*

1a. Pectoral fin rays more than 14; predorsal scales more than 9; lateral body with 6 oblique bands; caudal fin base with an oblique band ····· 2

2a. Anal fin rays I/7; longitudinal scale rows 28-29; transverse scale series 9-10; lateral blotches as broad as their interspaces; chin with many vermiform bars; caudal fin membrane with 5-9 vertical black lines ····· *H. hoevenii*

2b. Anal fin rays I/8; longitudinal scale rows 33-35; transverse scale series 11-12; lateral blotches about 1.5-2 times of their interspaces; chin without any dark marks; caudal fin membrane without any line or spot ····· *H. crassa*

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